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Amino Acid Composition of κ-Casein and Terminal Amino Acids of κ- and Para-κ-Casein[1]

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The amino acid composition of κ-casein is reported. Carboxypeptidase liberates Ser, Thr, Ala and Val from κ-casein, Leu and Phe from para-κ-casein. These Leu and Phe residues may be involved in the linkage (probably an ester linkage) split by rennin during its action on κ-casein.

INTRODUCTION

Wake (1) described the preparation of κ-casein, but no amino acid composition was available until now. Hipp *et al.* (2, 3) recently reported a method for fractionating α-casein into components designated α_1-, α_2-, and α_3-caseins; these authors have indicated the amino acid composition of all these fractions.

We now report the amino acid composition of κ-casein, its terminal amino acids, and also those of para-κ-casein. A short discussion will be included concerning the comparison of the new data with some previously reported results and the relation between κ-casein, para-κ-casein, and the caseino-glycopeptide obtained after rennin digestion of κ-casein.

MATERIALS AND METHODS

κ-Casein was prepared according to the method of McKenzie and Wake (4) and its caseino-glycopeptide according to Alais and Jollès (5); these two preparations appear homogeneous in sedimentation. The homogeneity of κ-casein has been discussed by Wake and Baldwin (6). Para-κ-casein was obtained by rennin digestion of κ-casein followed by the purification of the precipitate.

The amino acid composition has been determined on a total hydrolyzate (18 hr., at 110°, 6 *N* HCl) with a Technicon autoanalyzer according to the procedure of Piez and Morris (7). Tryptophan was determined according to Spies and Chambers (8).

The C-terminal amino acids were determined by the action of carboxypeptidase (pH 7.8; 10 mn., 37°, presence of diisopropyl phosphorofluoridate) and the N-terminal amino acids by the procedure of Sanger (9).

RESULTS AND DISCUSSION

The analytical results are summarized in Table I. They are expressed in grams/100 g. protein and in residues per mole of mol. wt. 26,000 ± 3,000 (10). The peptidic part accounts for 80%; the nonpeptidic part was estimated to 5%: 1.4% galactose, 2.4% *N*-acetylneuraminic acid, 1.2% galactosamine, and 0.217% P (5). Fifteen per cent of κ-casein escaped to analysis by the methods used until now; the same discrepancy has already been observed for α-casein and different caseino-glycopeptides (5). The cystine present in κ-casein is easily oxidized during the analysis (in absence of performic acid) and is titrated partially as cysteic acid. κ-Casein seems to have an amino acid composition similar to that of the α_3 component of Hipp *et al.* (3). Particularly noteworthy are the large differences for cystine, methionine, glycine, histidine, alanine, and tryptophan between κ-casein and α-casein

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TABLE I

AMINO ACID COMPOSITION OF κ-CASEIN

Amino acid	g./100 g. protein	Calculated residues/M.W. 26,000 ± 3,000
Asp	7.30	17
Thr	6.64	17
Ser	6.09	18
Glu	17.35	36
Pro	8.78	23
Gly	1.31	5
Ala	5.41	18
Cys	1.40	1
Val	5.10	13
Met	1.0	2
Ileu	6.14	14
Leu	6.08	14
Tyr	7.40	13
Phe	4.07	8
Lys	5.76	12
His	1.67	3
Arg	4.0	7
Try	1.05	1–2
NH_3		(17)
Total (g./100 g. protein)	96.55	223
(residues g./100 g. protein)	80.5	

(3). Our κ-casein has a higher content in hydroxyamino acids than the α_3 fraction.

Carboxypeptidase liberates Ser, Thr, Ala and Val from κ-casein; these amino acids are the same as those released from the caseinoglycopeptide (11) obtained after rennin digestion of κ-casein. For this reason this peptide seems to be situated at the C-terminal side of κ-casein, but it was not possible until now to decide if there are several peptide chains or if Ser, Thr, Ala and Val form the C-terminal sequence.

Carboxypeptidase liberates Leu and Phe by its action on para-κ-casein.

With Sanger's method, no N-terminal amino acids could be detected by the usual way for κ-casein, para-κ-casein, and the caseino-glycopeptide (treatment with FDNB at pH 8); the only DNP-amino acids obtained in a sufficient quantity were ε-DNP-lysine and *O*-DNP-tyrosine. The possible presence of DNP-galactosamine is under investigation.

By comparing the composition of κ-casein (about 5% glucidic and 80% peptidic part) and of its caseino-glycopeptide (28% glucidic and 72% peptidic part) (5, 11), it is possible to conclude that nearly all the sugars must be situated in the C-terminal part of κ-casein. As the Leu and Phe residues of para-κ-casein liberated by carboxypeptidase became accessible only after the reaction of rennin on κ-casein, they may be involved in the linkages split by this enzyme during its action on κ-casein. Some preliminary reduction experiments with $LiBH_4$ seem to indicate that this linkage may be an ester linkage.

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